PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Akira IDENO, et al.

Application No.: 10/511,098

Confirmation No.: 9139

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Docket No: Q83564

Group Art Unit: 1652

Examiner: Rebecca E. Prouty

For: EXPRESSION VECTOR, HOST, FUSED PROTEIN, PROCESS FOR PRODUCING FUSED PROTEIN AND PROCESS FOR PRODUCING PROTEIN

DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Akira Ideno, hereby declare and state:

THAT I am a citizen of Japan;

THAT I have received a Doctorate degree in Agricultural Science from KOBE University;

THAT I have been employed by Sekisu Chemical Co., Ltd. since 1992, where I hold a position as Research Scientist, with responsibility for sub-managing;

THAT I am an inventor of the above mentioned application;

THAT the following data was generated by my laboratory under my direction and supervision;

THAT I have read the Office Action dated August 9, 2007, and understand the basis of the rejections set forth therein and the references cited by the Examiner, particularly Fersht et al. and Furtani et al.; and

THAT Fersht et al. and Furtani et al. fail to teach, suggest or motivate one of ordinary skill in the art Applicants' invention; and Fersht et al. and Furtani et al. fail to suggest or disclose the unexpected properties of Applicants' invention.

I. Experimental Goals

To directly compare Applicants' invention to Fersht et al. (WO 00/075346) as cited by the Examiner.

A TcFKfusion2 of an expression vector for fusion with a short type FKBP-type PPIase (TcFKBP18) was derived from hyperthermophilic archaebacterium *Thermococcus* sp. KS-1, taught in Example 1 of the present application, and an expression vector into which a GroE1191-345 fragment is introduced, which is disclosed in Fersht et al., was prepared and expression levels were determined.

Protein production in conventional expression systems using *Escherichia coli* is difficult, since expressed proteins auto-aggregate and kill host cells. Table 1 shows the properties of the proteins studied.

Table 1

Protein name	Property	Molecular weight
Anti-hen egg white lysozyme scFv antibody fragment (mouse) (scFv antibody fragment)	Aggregative protein	26kD
Hepatitis C virus core anitigen (HCo)	Toxic protein	21kD
Serotonin receptor (SerR)	Toxic protein	46kD
Endothelin A receptor	Toxic protein	47kD

II. Materials and Methods

1. Construction of the expression vector for TcFKfusion2

Vector TcFKfusion2 was obtained following the same method described in Example 1 of the Applicants' specification.

2. Construction of expression vector of GroEl(191-345)fusion

The TcFKfusion2 obtained in (1) was utilized for the construction of an expression vector. A PPIase gene was removed from the TcFKfusion2 by subjecting the TcFKfusion2 to treatment with NcoI/SpeI. Next, a GroEL191-375 DNA was introduced into the vector by ligating the GroEL191-375DNA to the TcFKfusion2. Thus, the expression vector GroEl(191-345) fusion in which a PPIase gene on a TcFKfusion2 is substituted for a GroEL191-375 was created.

3. Construction of expression vector of fused protein

An expression system of each fused protein was constructed by introducing a gene encoding a scFv antibody fragment, HCc, SerR and ETAR, into the obtained TcFKfusion2 and GroEl(191-345)fusion.

4. Expression of each fused protein

An expression vector of each fused protein was transformed into *E. coli* BL21 (DE3) strain, and colonies were transferred to 200 mL of a 2 x YT culture medium. After culturing (stirring at 110 rpm) at 35°C for 7 hours, IPTG was added, and culturing was continued over night. Cells were harvested by centrifugation (10000 rpm x 10 min). The cell pellets were suspended in 10 mL of a 25 mM HEPES buffer (pH 6.8) containing 1 mM EDTA.

Cell solutions were disrupted by sonication, and centrifuged to separate the supernatant (soluble fraction) and the precipitated part (insoluble fraction). The obtained 10 μL of the soluble fraction and 5 μL of the insoluble fraction are subjected to SDS-PAGE, and the resultant was stained with Coomassie Brilliant Blue (CBB).

III. Results

1. Expression in TcFKfusion2 system

A CBB stained image of an SDS-PAGE of a soluble fraction (3) and an insoluble fraction (I) in each fused protein expression system using a TcFKfusion2 is shown in Fig. 1.

As shown in Fig. 1(a), a band corresponding to a fused protein of a scFv antibody fragment and a PPIase was observed in the vicinity of 42 kDa both in a soluble fraction (S) and an insoluble fraction (1) in a fused protein expression system. According to Figure 1, about 59% of the proteins are expressed in the soluble fraction of the cytoplasm. The level of expression of the fusion protein contained in the soluble fraction (3) was about 14% total soluble protein.

As shown in Fig. 1(b), a band corresponding to a fused protein of a HCc and a PPIase was observed in the vicinity of 39 kDa in a soluble fraction (S) in a fused protein expression system.

As shown in Fig. 1(c), a band corresponding to a fused protein of a SerR and a PPIase, and a band corresponding to a fused protein of a ETAR and a PPIase, were observed in the vicinity of 70 kDa in a soluble fraction (S) in a fused protein expression system.

In addition, fused proteins were observed by immunostaining.

2. Expression in GroEl(191-345)fusion system

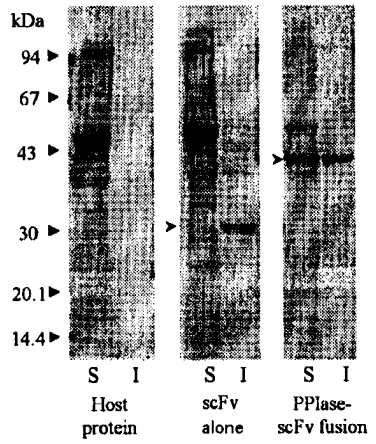
A CBB stained image of an SDS-PAGE of a soluble fraction (S) and an insoluble fraction (I) in each fused protein expression system using a GroEl(191--345) fusion is shown in Fig. 2.

A fused protein of a GroEll91-375 fragment and a HCc, a scFv, an endothelin A or a serotonin receptor, corresponds to bands of 41kDa, 46kDa, 67kDa or 66kDa. According to Fig. 2 however, a band corresponding to each fused protein was not observed both in a soluble fraction (S) and an insoluble fraction (I). A band is detected in the vicinity of 26 kDa of a molecular weight in an insoluble fraction, which is a GroE1191-375 fragment. A fused protein was not detected by immunostaining. The results are summarized in the following table.

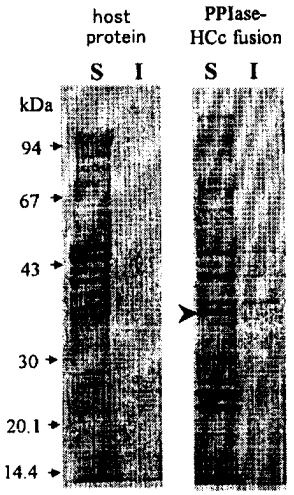
Table 2

Protein Type	TcFKfusion 2 system	GroEl (191-345)fusion system
scFv antibody fragment	+[1]	-
HCc antigen	+	-
Serotonin receptor	+	-
Endothelin A receptor	+	-

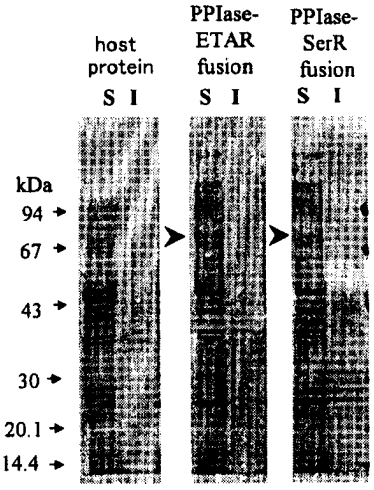
[1] "+" means expression of the fused protein was observed; "-" means expression of the fused protein was not observed.



A. Expression of scFv



B. Expression of HCc



C. Expression of SerR, ETAR

Fig. 1. A CBB stained image of SDS-P7GE of a soluble fraction (S) and an insoluble fraction (r) in each fused protein expression system using a TcFKfusion2.

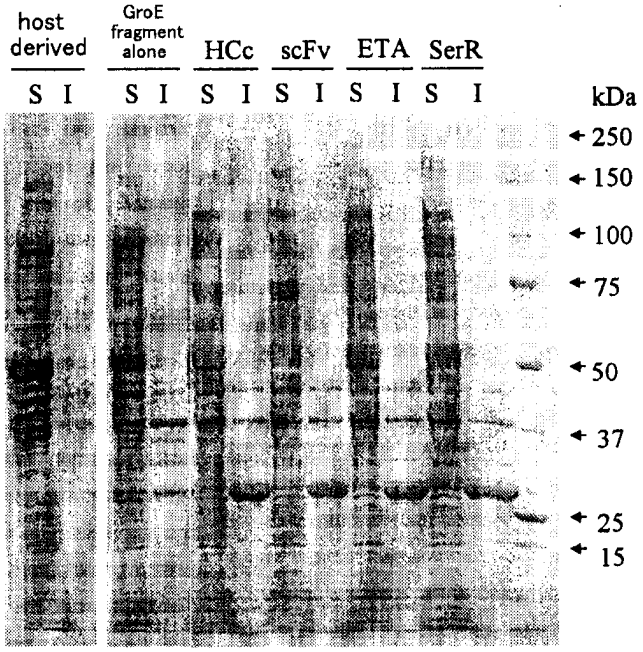


Fig. 2. A CBB stained image of SITS-PAGE of a soluble fraction (S) and an insoluble fraction (I) in each fused protein expression system using a GroEl(191-345) fusion.

IV. CONCLUSION

Based on the experiments set forth herein, and in view of the cited references, Applicants' vector is not disclosed or suggested by the cited literature. The cited references fail to disclose the elements of Applicants' invention, particularly a vector encoding a fusion protein wherein molecular chaperone activity is retained and a soluble fusion protein is obtained.

Fersht et al. and Furtani et al. fail to suggest or motivate one of skill in the art to modify the references to make Applicants' invention. Fersht et al. and Furtani et al. also fail to suggest or disclose the unexpected properties of Applicants' invention. In addition, there is no reasonable expectation of success that modification of Fersht et al. and Furtani et al. will yield Applicants' invention.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 8th, Nov, 2007

Akira Ideno

Akira Ideno